

Application No. 10/665,460  
Reply to Office Action of February 26, 2007

Docket No.: 05500-00154-US

**Amendments to the Specification:**

Please amend the Specification, without prejudice, as follows:

At pages 56-58, please amend paragraphs [00125] through [00130] (which appear in the published version of the application as paragraphs [0128] through [0134]), as shown below:

[00125] The overall increase in the leucine, phenylalanine and glutamic acid content of the Cry proteins is described below for the Cry9Ca1 toxin. Although this example is carried out on the Cry9Ca1 protein and the cry9Ca1 gene, its teaching is applicable to all the Cry toxins and all the cry genes. This teaching applies in particular to all the Cry toxins the sequence of which is known and filed in the Genbank database.[:]

~~www.ncbi.nlm.nih.gov/Genbank/index.html.~~

The Genbank accession numbers for the cry genes are also available on the internet following site:

~~www.biol.susx.ac.uk/Home/Neil\_Crickmore/Bt/index.html.~~

[00126] This teaching also applies to all the Cry toxins and cry genes, the sequences of which are not disclosed on Genbank.

[00127] Unlike the strategies described in Examples 1 to 4, the aim is not to modify a precise region of the toxin so as to integrate amino acids recognized by pepsin, but to increase, overall, the number of these sites by increasing the amount of leucine, of phenylalanine and of glutamic

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acid in said toxin. This strategy makes it possible to make the Cry toxin more sensitive to pepsin by increasing the percentage of residues recognized by pepsin. Glutamic acid (E; Glu) preferentially substitutes for aspartic acid (D; Asp), phenylalanine (F; Phe) preferentially replaces tryptophan (W; Trp) and leucine (L; Leu) preferably replaces valine (V; Val) or isoleucine (I; Ile). This strategy require the creation of a three-dimensional model for the activated Cry9Ca1 toxin, created from the primary sequence of the protein by comparison with the three-dimensional structures of Cry1Aa1 and Cry3Aa1. The model was created using the Swiss-Model Protein Modelling Server (Peitsch, 1995; Peitsch, 1996; Guex and Peitsch, 1997). The server address is as follows:

~~[www.expasy.ch/swissmod/swiss-model.html](http://www.expasy.ch/swissmod/swiss-model.html)~~

[00128] Preferably, the substitutions should reach a maximum level of 25%. The activated Cry9Ca1 toxin contains 31 aspartic acids, 9 tryptophans and 47 valines. There are naturally 26 glutamic acids, 35 phenylalanines and 62 leucines. Taking into account a maximum substitution of 25% for each of the amino acids, the relative ratios are as follows:

| Amino acid | Number of residues in native Cry9Ca1 | Number of residues in modified Cry9Ca1 |
|------------|--------------------------------------|--|
| Asp (D)    | 31                                   | 24                                     |
| Glu (E)    | 26                                   | 33                                     |
| Trp (W)    | 9                                    | 7                                      |
| Phe (F)    | 35                                   | 37                                     |
| Val (V)    | 47                                   | 36                                     |
| Leu (L)    | 61                                   | 72                                     |

[00129] The substitution of isoleucine (I; Ile) with leucine can also be envisioned instead of or in addition to the substitution of valine with leucine. There are naturally 27 isoleucines in the Cry9Ca1 toxin. Taking into account a preferential degree of substitution of 25%, it is sufficient to replace 6 isoleucine residues with leucines.

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[00130] It is possible to modify the sequence of the cry9Ca1 gene as shown below. The only aim of the demonstration below is to illustrate the example, and it does not in any way limit the scope of the invention. This demonstration relates to aspartic acid, tryptophan and valine residue replacement. Those skilled in the art can very easily adapt this approach to any other cry gene, the sequence of which would be known, and in particular from the sequences available on Genbank and the their accession numbers of which are mentioned on the following site:

~~www.biol.susx.ac.uk/Home/Neil\_Crickmore/Bt/index.html.~~